

Figure 1:

Amino acid sequences of Cpn60 and Cpn10:

SEQ ID No 1: Cpn10 (encoded by nucleotides pos. 458-751 of Figure 2):

MKIRPLHDRIVVRRKEEETATAGGIILPGAAAEEKPNQGVVISVGTGRILDNGSVQALA
VNEGDRVVFVFGKYSGQNTIDIDGEELLILNESDIYGVLEA

SEQ ID No 2: Cpn60 (encoded by nucleotides pos. 800-2446 of Figure 2):

MAAKDVLFGDSARAKMLVGVNILADAVRVTLGPGRNVVIEKSFGAPIITKDGVSVA
AREIELKDKFENMGAQMVKEVASQANDQAGDGTTATVLAQAIISSEGLKSVAAGMN
PMIDLKRGIKD KATAAVVAAIKEQAQPCLDTKAIAQVGTISANADETVGRLIAEAMEKV
GKEGVITVEEGKGLEDELDVVEGMQFDRGYLSPYFINNQEKM VEMENPLILLVDKK
IDNLQELLPILENAKSGRPLLIV AEDVEGQALATLVVNNLRGTFKVA AVKAPGFD
RRKAMLQDLAILTGGQVISEELGMSLETADPSSLGTASKVVIDKENTVIVDGAGTEAS
VNTRVDQIRAEIESSTDYDIEKLQERVAKLAGGVAVIKVGAGSEM EMEK KDRVD
DALIHATRAAAVEEGVVAGGGVALIRALSSVTVVGDNEDQNVGIALALRAMEAPIRQI
AGNAGAEGSVVVDKVKGTSFGFNASTGEYGD MIAMGILDPAK VTRSSLQAAASI
AGLMITTEAMVADAPVEEGAGGMPDMGGMGGMGGMPGMM

Figure 2:

SEQ ID No 3: DNA coding for Cpn60 and Cpn10:

Cpn10, pos. 458-751

Cpn60, pos. 800-2446

atcaaaaaatgcagcaaggacagattcctgcccaagaattgcagaaggttctttagcactggccggcgcttattattaacgccgg
gttttgtcactgatgcgtgggttttacattactcgccccgcacgcgtaaagcgttgtccataaggtgattgcatttattaccctc
gcatgatgactgcaagcagcttcaagcgacggtagtttcaggaaggctcgtaaagatgtacattgcacactgactcgcaaagca
gtcatgaaaaaatcacaattgaaggcgaatataccaaagacgataagttaggtattttcggctagccgttgaatcctagtaaaagccc

Figure 3:

SEQ ID No 4: Amino acid sequence of esterase cloned from *Oleispira antarctica* (EstRB8):

EstRB8 (encoded by nucleotides 1145 to 2143 Frame 2 of Figure 4) 333 aa

MKNTLKSSSRFSLKQLGTGALISSLFFGGCTTQQDNLYTGVMSLARDSAGLEVKA
SAGDVNLTYMERQGSKDNAESVILLHGFSADKDNWILFTKEFDEKYHVIAVDLAG
HGDSEQLLTTDYGLIKQAERLDIFLSGLGVNSFHAGNSMGGAIASIYSLSHPEVKSL
TLIDAAGVDGDTESEYYKVLAEGKNPLIATDEASFYRMGFTMTQPPFLPWPLRPSLL
RKTLARAЕINNKIFSMLKTKERLGMTNFQQKIEVKMAQHPLPTLIMWGKEDRVLD
VSAAAFAKKIIPQATVHIFPEVGHLPMVEIPSESAKVYEEFLSSIK

Figure 4:

SEQ ID No 5: DNA fragment from plasmid pBK1Est coding for esterase of *Oleispira antarctica* (EstRB8):

Nucleotide positions 1-100 correspond to reverse complement of positions 1196-1121 and 3799-3939 correspond to reverse complement of 1043-952 of pBK-CMV vector (Stratagene).

Positions 101-105 are *Bam*H I – *Sau*3A I fusion and positions 3795-3798 are *Sau*3A I-*Bam*H I-fusion.

cgttatttattacacggttctgctgataaagataactggattcttaccaaagaattcgataaaaatcatgttatcgctgcattt
gcgggacatggcgattcagaacaattattaacgactgattacggctcataaaacaagccgagcgtagatcttctatctggcttagg
ggtaactcattcacatcgccgtaattcaatggggggctatcagcgcaatctacagttgagtaccaggagaagttaaaagtctt
acattgatcgatcgagggtcgatggcgatactgaaagcgaataactacaaagtttggcagaaggtaagaatctttaattgcaact
gatgaagcaagtttgaataccgcatgggttcaccatgactcagcctccttaccttgccactaagaccccttattacgtaaaacg
ctagcccgtgccgagatcaataacaaaattttccgatgctgaaaaccaaagaacgtttaggaatgactaacttcaacagaaaattg
aagtggaaaatggctcaacatccattccaacactgattatgtgggcaaagaagatcgcttgcgtatccgcagcagcccttc
aaaaaaaataattccacaagcaactgttcatattttcctgaagtaggcacctacatggtagaaattcttagtggctaaagttat
gaagagtttgcctctattaaataagagcacataatcatgactgacttataaacagccaagcattaaatgttggcttattttatgg
ccaaattattcaacgaccaagctctggtaaaatcgactgggttgcattcaacagcaacaaacgtgaaatacccgtaatcg
cattttctgattatcaaaatacatactttccaccaggcatattaactcaactttaaactcgtccgcctacctctataacactggcagtcaatt
cgacaatggtacctgcggAACAGGATGCTTAAATCGATTGATCAGTCGTACGCTGACGGTTACGATGCTTGTGAGAAAAACGAGTCGT
GCAATAAAAGAAAACCTCATCCACTGCATTGCACTGCCACCAGAATAACGTATGATGATTGTTGTCTGGAAATACCGCTTGA
AATAGGGTTTGTACCGCTTGCCTGCGCAATAATCTCTGCTAAGAGTTGGGATGGCATACTAAACTCGCTGATTAAGGATTA
ATAATAATAGTTAACAGTATATTGAACTGAGGGCTGAGAAGAACTCTAATACCTCTGAGAACTTTGAGGCCGCTAGAGAGAAAAGACCA
GTGATAATATTCACTTGCCATGAGAGCTTATCATGAAAGCCTGCTTAAATCAATCATTATATTCACTTAAATTGAAATAACCAAT
ATATTCACTATATAATTCAACTACCCTTACTCAGACTTCCCGCGCATAGCGCAAACAATCAACGCAAGTTCTACCGTTGCA
GCTGCAACACATGCCCTAGCGCTAAAGTAGCACGCACAACACTGGCCAGTCGTACTAGCCCCTTGCATTGTCAGACGAGC
AACAGCGCTTAAACTACCTAAATTCTAACCAACCACCATGGCTTCCACAAACTCAAAAAACTCGTCAAATCCGCTTGC
ATTGACATAGATCTAATCGATTCAAACCCGATTCAAGCGCTTAAACGCAAGACTTCTACCTGCACTGACCA
ATATGCAAGCGCGGGCGGAAGAGCTGCCATTGATCGATCAAGAAGAAGGGAGCAGCAAAGAGGAAACAATCAAAAAGAGGAG
GCAATCAAATAAAACGAGTTATTGAGGATTAAATTAAACAGGTATATTAAACCTCTCGTAGTAAACAATGACTGTATTACCAA
AAATAATAGAGGTACCATGTCACACATCTGGTTGAAGTACCAAAGATTGAAGTATTAAACCGTCAAATGAAAATACTGCC
GACACTAGGCATTCAAATTACAGAAATTGGCGATGATTATCAGTCGTACGCCAGCAGTCACGTCAGGCCATTGGACTG
ATTCACTGGCGGCTCAAATGTATTGCTGGCAGAAACACTGGCAGCATGGCAGCTAAGTCGTATTGCTCAAGAATATTGTG
CCAAGAAAATTACGCCAACACATCGCGGTTCGTTCCGGCATAGTCAGTCACAGCAACGCTAGTACACAAAGGAAGAAC
CCAGATTGGAAATTGCGATCGTTAACGATCAAAGATTCAAAGCTCTCGAGAGTACTTCTAGAGCGGCCGCGGGCCATCG
TTCCACCCGGGGGGTACCAAGTGTACCAAATTGCCCTATAGTGAGTCGTATTACAATTCACTGGCGTCGTTAC

Figure 5:

Amino acid sequences expressed from vector pBK1CpnEst: - the co-expression of fragments encoding native chaperonines with the esterase gene (EstRB8), all from *Oleispira antarctica*

SEQ ID No 6: cpn10 (nucleotides 113 to 403: Frame 2 of Figure 6) 97 aa:

MKIRPLHDRIVRRKEEETATAGGIILPGAAAEEKPNQGVVISVGTGRILDNGSVQALA
VNEGDVVVFGKYSGQNTIDIDGEELLILNESDIYGVLEA

SEQ ID No 7: cpn60 (nucleotides 455 to 2098: Frame 2 of Figure 6) 548 aa:

MAAKDVLFGDSARAKMLVGVNILADA VRVTLGPKGRNVVIEKSGAPIITKDGVSV
AREIELKDKFENMGAQMVKEVASQANDQAGDGTTATVLAQAIISEGLKSVAAGMN
PMIDLKRGIKD KATAAVVAAIKEQAQPCLDTKAIAQVGTISANADETVGRLIAEAMEKV
GKEGVITVEEGKGLEDELDVVEGMQFD RGYLSPYFINNQEKM T VEMENPLILLVDKK
IDNLQELLPILENAKSGRPLLIV AEDVEGQALATLVVNNLRGTFKVA AVKAPGFGD
RRKAMLQDLAILTGGQVISEELGMSLETADPSSLGTASKVVIDKENTVTVDGAGTEAS
VNTRVDQIRAEIESSTS DYDIEKLQERVAKLAGGVAVIKVGAGSEM EMEK KDRVD
DALHATRAAVEEGVVAGGGVALIRALSSVTVVGDNEDQNVGIALALRAMEAPIRQI
AGNAGAEGSVVVDKVKS GTGSFGFNASTGEY GDMIAMGILDPAK VTRSSLQAAASI
AGLMITTEAMVADAPVEEGAGGMPDMGGMGGMGGMPGMM

SEQ ID No 8: estRB8 (nucleotides 2579 to 3577: Frame 2 of Figure 6) 333 aa:

MKNTLKSSSRFSLKQLGTGALISSLFFGGCTTQQDNLYTGVM SLARDSAGLEV KTA
SAGDVNLTYMERQGSDK DNAESVILLHGFSADKDNWILFTKEFDEKYHVI AVDLAG
HGDSEQLLTDYGLIKQAERLDIFLSGLGVNSFH IAGNSMGGAI SAIYSLSHPEKVKSL
TLIDAAGV DGDTESEYYKVLAEGKNPLIATDEASFEYRMGFTMTQPPFLPWPLRPSLL
RKTLARA EINNKIFS DMLKT KERLGMTNFQQKIEVKMAQHPLPTLIMWGKEDRVLD
VSAAA AFKKIIPQATVHFPEVGHLP MVEIPS ESAK VYEEFLSSIK

Figure 6:

SEQ ID No 9: pBK1CpnEst: - the fusion of native chaperonine-coding fragments with esterase of *Oleispira antarctica* (EstRB8)

The DNA fragment coding for Cpn10 and Cpn60 is flanked by *SacI* site (pos. 69-75) and *SalI* site (encoded by pos. 2138-2143 of Figure 7):

Nucleotide positions 1-75 correspond to reverse complement of positions 1196-1121 and positions 5233-5273 correspond to reverse complement of 1043-952 of pBK-CMV vector (Stratagene)

Small letters – the Cpn10-Cpn60 encoding fragment.

Capital italics – fragments of vector pBK-CMV

Capital letters – fragment coding for EstRB8 from plasmid pBK1Est

gttgggtcggttcgtgaaatggaaatgaaagagaagaaagaccgtgtgacgatgcactcatgcaactcgcgcagcggttgaagaag
gtgttgtcggtgggtggttgtgcgttgcgcactctcttcagtaaccgttgtgtgataacgaagatcaaaacgtcggtattgcat
tggcacttcgtgcgtatccgtcaaattcgccggtaacgcagggtgtgaagggtcagtgggtgtgataaaagtgaaatctg
gcacaggtagcttggtttaacgccacagggtgagttatggcgatattgcgtatgggtatttagaccctgcaaaagtacgcgttc
atctctacaagccgcggcgatcgccagggtgtatgcgtatgcgtatgcgtatgcgtatgcgtatgcgtatgcgtatgcgtatgcgt
gtatgcgtatggcgatgggtggaaatggcggtatgcgtatgcgtatgcgtatgcgtatgcgtatgcgtatgcgtatgcgtatgcgt
GTCGACATATTCAAGATAAAAGATGCCTTCACTGACATCAGTCACCAACAATCAAT
CAAACACCAATACCAATCGAAAAACTCATAAAAGTAGCCGATCACCAAAATCCC
AAAAGCGTTCAAAAATGAAACGAGCACGTACACAAAATCAATTATACGCTAA
CGAACCAAGGTCAAACCTATCGTTTTGAGCACGTTGTTCCACTAATGAAAGA
GAAAAGTCGTTAATTCACTGGCTTTGGCGTATCCGACCTTCACATAGAAATT
GTAATGGCATGCTACTGGCCTTAAAAAGAATCAGTTAATTGAAGAACCTCGCT
TATCTCAGCCATTACCGCTGTAGCCGAATTGCGCTTATCCTCAGCCATGATTAAA
CTGACGCCAATTAAATATAAGACATACTAATTAAATAACTCCCTTAATTGAGAAGAA
TAATGAAAAACACACTCAAATCCTCATCACGTTAGTCTGAAACAAACTCGGCAC
CGGCCTCTGATTATCTCCAGTTGTTCTCGGTGGTGCACCACACACAAAG
ATAATTATACACAGGGTTATGTCTCTGCGAGAGACAGCGCTGGCCTAGAAGT
TAAAACAGCCTCTGCCGGTGACGTCAATCTTACTTATATGGAACGCCAAGGCAGT
GACAAAGATAATGCCGAAAGCGTTATTTATTACACGGTTCTGCTGATAAAAG
ATAACTGGATTCTTTACCAAAGAATTGATGAAAAATATCATGTTATCGCTGTC
GATTAGCGGGACATGGCGATTAGAACAAATTAAACGACTGATTACGGTCTCA
TAAAACAAGCCGAGCGTTAGATATCTTCTTATCTGGCTAGGGTTAACTCATT
CACATCGCCGGTAATTCAATGGGGGGCTATCGCAGCAATCTACAGTTGAGTC
ACCCAGAGAAAGTTAAAAGTCTTACATTGATCGATGCAGCAGGTGTCGATGGCG
ATACTGAAAGCGAATACTACAAAGTTGGCAGAAGGTAAGAACCTTTAATTGC
AACTGATGAAGCAAGTTGAATACCGCATGGTTCTCACCAGTACTCAGCCTCCT
TTCCTACCTTGGCCACTAAGACCTTCTTATTACGTAAAACGCTAGCCGTGCCGA
GATCAATAACAAATTTCGATATGCTGAAAACCAAGAACGTTAGGAATG
ACTAACTTCAACAGAAAATTGAAGTGAAAATGGCTAACATCCATTGCCAACAC
TGATTATGTGGGCAAAGAAGATCGCGTTCTGACGTATCCGCAGCAGCGGCCTT
CAAAAAAAATAATTCCACAAGCAACTGTTCATATTTCCTGAAGTAGGCCACCTA
CCTATGGTAGAAATTCTTAGTGAAAGCGCTAAAGTTATGAAGAGTTGTCCT
CTATTAAATAAGAGCACATAATCATGACTGACTTATAAACAGCCAAGCATTAAA
ATGCTTGGCTGTTATTAAATGCCAAATTATTCAACGACCAAGCTCGCGTAA

AATCGCAGTGGGTTCTGTTCATCAACAGCAACAAACGTGAAATAACCCGTA
ATCGCATTTCCTGATTATCAAAATACATACTTCCACCAGCATATTAACCTAAC
TTTAAACTCGTCCGCCCTACCTCTATAACACTGGCAGTCATTGACAATGGTAC
CTGCGGGAACAGGATGCTAAAATCGATTGATCACTGCTGACGGTTACGATGCT
TTGTCGAGAAAAACGAGTCGCTGCAATAAAAGAAACCTCATCCACTGCATT
GCAGTGCCACCGAATAACGTATCATGATGATTGTTCTCTGGAAATACCGCTTT
AGAAATAGTGGTTTGATA CGCGCTTCGCTGCGCAATAATATCTTCTGCTAA
GAGTTGCGGATGGCATA CATAACTCGCTGATTAAGATTAATAATAAGTTA
ACAGTATATTGA ACTGAGGGTCTGAAGAACTCTAACCTCTGAAGAACTTGAG
GCCGCTAGAGAGAAAAGACCAGTGATAATATTCA TCTGCCATGAGAGCTTATC
ATGAAAGCCTGTGCTAAAATCAATCATTATATTATTCA TCTTAATTGAAATAA
TACCAATATATTCA TATAATTCA CACTACCCCTATCTCACTAGACTTCCCAC
GCATAGGCGAAACAATCAACGCAAGTTACA AAGCGGTTGCTGCAACAC
ATGCCCTAGCGTCTAAAGTAGCACGCACAACACTGGCCAGTCGTACTAGCCCCT
TGCGATTGCGACAGACGAGCAACAAGCGCTATTAAACTACCTAAATTCTAAC
ACCACCA TTGGTCTTCCACAAACTCAAAAAACTCGTCAAATCCGCTTGCAATT
TAAACGCGATGACATAGATCTAATCGATTATCAAACCCGCATTCAAGCGCTCATT
AAAAACGCACCACTGGCAAGAAGTTCTACCTGCACTGACCAATATGCAAGCGGC
GGCGGAAGAGCTGCCTTGATCGATCAAGAAGAAGGGAGCAGCAAAGAGGAAA
ACAATCAAAAAGAGGAGAGCAATCAAATAAAACGAGTTATTGAGGATTAAAT
TTTAAAACAGGTATATTAAATACCTCTCGTAGTAAACAATGACTGTATTACAC
AAAAATAAAATAGAGGTATACCATGTCAAACATCTGGTTGAAGTACCAAAGATTG
AAGTATTAAACCGTCAAATGGAAAATCTGCCTGCAGCAACTAGGCATTCAAAT
TACAGAAATTGGCGATGATTATATCACTGGCACAATGCCAGCAGATGCACGTACC
TTCCAGCCAATGGGACTGATTCA TGGCGGCTCAAATGTATTGCTGGCAGAAACAC
TGGGCAGCATGGCAGCTAACTGCTGTATTAAATTGTCTCAAGAATATTGTGTTGG
CCAAGAAATTACGCCAACACATACGCGGTGTTCCGGCATAGTGA CTGGC
ACAGCAACGCTAGTACACAAAGGAAGAACCTCCCAGATTGGAAATT CGC ATC
GTTAACGATCCAAGAATTCAAAGCTCTCGAGAGTACTCTAGAGCGGCCGCGGG
CCCATCGATTCCACCCGGGTGGGTACCGAGGTAA GTGTACCCATTGCCCTATAGT
GAGTCGTATTACAATTCACTGGCCGTGTTAC

Figure 7:

Amino acid sequences expressed from vector pBK1CpnSREst: - the co-expression of the stabilized single ring mutant chaperonin with the esterase gene (EstRB8) from *Oleispira antarctica* (cpn10::stabilized single ring mutant Glu461Ala/Ser463Ala/Val464Ala::est)

SEQ ID No 10: cpn10 (nucleotides 113 to 403: Frame 2 of Figure 8) 97 aa:

MKIRPLHDRIVVRRKEEETATAGGIILPGAAAEEKPNQGVVISVGTGRILDNGSVQALA
VNEGDVVVFGKYSGQNTIDIDGEELLIENESDIYGVLEA

Below – ***Capital bold letters*** are the mutations introduced

SEQ ID No 11: stabilized single ring mutant of cpn60 (nucleotides 455 to 2098: Frame 2 of Figure 8) 548 aa:

MAAKDVLFGDSARAKMLVGVNILADA VRVTLGPKGRNVVIEKSFGAPIITKDGVSVA
AREIELKDKFENMGAQMVKEVASQANDQAGDGTTATVLAQAIISEGLKSVAAGMN
PMIDLKRGIDKATAAVVAAIKEQAQPCLDTKAIAQVGTISANADETVGRLIAEAMEKV
GKEGVITVEEGKGLEDELDVVEGMQFDRGYLSPYFINNQEKMVTEMENPLILLVDKK
IDNLQELLPILENAKSGRPLLIVVAEDVEGQALATLVVNNLRGTFKVAAVKAPGFGD
RRKAMLQDLAILTGGQVISEELGMSLETADPSSLGTASKVVIDKENTVIVDGAGTEAS
VNTRVDQIRAEIESSTS DYDIEKLQERVAKLAGGVA VIKVGAGSEMEMKEKKDRVD
DALHATRAAVEEGVVAGGGVALIRALSSVTVVGDNEDQNVGIALALRAMEAPIRQI
AGNAGAAGAAVVDKVKSGTGSFGFNASTGEYGDMIAMGILDPAKVTRSSLQAAASI
AGLMITTEAMVADAPVEEGAGGMPDMGGMGGMGGMPGMM

SEQ ID No 12: EstRB8 (nucleotides 2579 to 3577: Frame 2 of Figure 8) 333 aa:

MKNTLKSSSRFLKQLGTGALISSLFFGGCTTQQDNLYTGVMMSLARDSAGLEVKTASAGDVNLTYMERQGSDKDNAESVILLHGFSADKDNWILFTKEFDEKYHVIAVDLAGHGDSEQLLTDYGLIKQAERLDIFLSGLGVNSFHIAGNNSMGGAIYSLSHPEKVKSLLTLIDAAGVGDGTESEYYKVLAEGKNPLIATDEASFYRMGFTMTQPPFLPWPLRPSLL

RKTLARAЕINNKIFSMLKTKERLGMTNFQQKIEVKMAQHPLPTLIMWGKEDRVL
VSAAAAFKKIIPQATVHIFPEVGHLPMVEIPSЕSAKVYEEFLSSIK

Figure 8:

SEQ ID No 13: DNA sequence of vector pBK1CpnSREst: the expression cassette for the co-expression of the stabilized single ring mutant chaperonin with the esterase gene (EstRB8) from *Oleispira antarctica* (cpn10::stabilized single ring mutant Glu461Ala/Ser463Ala/Val464Ala::est)

Nucleotide positions 1-75 correspond to reverse complement of positions 1196-1121 and positions 5233-5273 correspond to reverse complement of 1043-952 of pBK-CMV vector (Stratagene)

DNA fragment coding for Cpn10 and Cpn60 is flanked by *SacI* site (pos. 69-75) and *SalI* site (pos. 2138-2143).

In the DNA sequence:

Small letters – the Cpn10-Cpn60 coding fragment,

Capital italics – fragments of vector

Capital letters – fragment coding for EstRB8 from plasmid pBK1Est

Capital bold letters = introduced mutations

ACAGGAAACAGCTATGACCTTGTGATTACGCCAAGCTCGAAATTAACCCTCACTAAAGGGA
ACAAAAGCTGGAGCTCtaatacttggatccaacagttggagagtctagcaaatgaaaatccgtccattacatgatcgatt
gttgcgtaaagaagaagagaccgcaactgcgggttgttattttaccggcgctgcggcagaaaaaccatcaagggttgt
tatctctgtgggtactggccgtattctgataatggtcagtgcacgcgtggcggttaacgaaggcgatgttgtcgaaaaatc
aggtaaaaactatcgatatcgatggtaagaattattgattttgaatgaaagtgatatacgccgttttagaagcttaattattacactca
ctttttattnaacctacaaaatttaaggaaagatcatggctgctaaagacgtatttttgtatagcgcacgcgaaaaatgttgttaggt
gtaaacattttagccgacgcagtaagagttaccctaggacctaaggtcgtaacgttgtatagaaaaatcatttgtgcaccgatcatcac
caaagatggtgtttctgttgcgctgtaaatcgaaattgaaagacaattcgaaaacatggcgacagatggtaaggaagttgcattctca
agccaaacgaccaagccgggtacggcacaacgacagcgactgtactgcacaggcgattatcagcgaaggctgaaatctgtgcgg
ctggcatgaatccaaatggatctaaacgttgtattgataaagctacggctgctgttgtccattaaagaacaagctcagccttgctt

CCTTCCTACCTGGCCACTAAGACCTCTTATTACGTAAAACGCTAGCCCGTGC
CGAGATCAATAACAAAATTTCGATATGCTGAAAACCAAAGAACGTTAGGA
ATGACTAACTTCAACAGAAAATTGAAGTGAAAATGGCTAACATCCATTGCCAA
CACTGATTATGTGGGGCAAAGAACGATCGCGTTCTGACGTATCCGCAGCAGCGGC
CTTCAAAAAAAATAATTCCACAAGCAACTGTTCATATTTCCTGAAGTAGGCCAC
CTACCTATGGTAGAAATTCTAGTGAAAGCGCTAAAGTTATGAAGAGTTTGT
CCTCTATTAAATAAGAGCACATAATCATGACTGACTTATAAACAGCCAAGCATT
AAAATGCTTGGCTGTTATTTAATGCCAAATTATTCAACGACCAAGCTCTGCG
GTAAAATCGCAGTGGGTTCTGTTCATCAACAGCAACAAACGTGAAATACCC
CGTAATCGCATTTCGATTATCAAAATACATACTTCCACCAGCATTTAACTT
CAACTTTAAACTCGTCCGCCCTACCTCTATAACACTGGCAGTCAATTGACAATG
GTACCTGCGGGAACAGGATGCTTAAATCGATTGATCACTGCTGACGGTTACGA
TGCTTGTCGAGAAAAACGAGTCGCTGCAATAAAAGAAACCTCATCCATCCACTG
CATTGCAGTGCCACCGAATAACGTATCATGATGATTGTTGTCTGGAAATACC
GCTTAGAAATAGGTTTGATACGCGCTTCGCTGCGCAATAATATCTCT
GCTAAGAGTTGCGGATGGCATACATAAACTCGCTGATTAAGATTAATAATAAAAT
AGTTAACAGTATATTGAAC TGAGGGCTGAAGAACTCTAACACCTCTGAAGAACT
TTGAGGCCGCTAGAGAGAAAAGACCAGTGATAATATTCAATCTTGCCATGAGAGC
TTATCATGAAAGCCTGTGCTTAAATCAATCATTATATTATTCATCTTAATTGA
AATAATACCAATATTCATATATAATTCAACACTACCCCTATCTCACTAGACTT
CCCGCGCATAGGCGAAACAATCAACGCAAGTCACAATAAGCGGTTGCTGC
AACACATGCCCTAGCGTCTAAAGTAGCACGCACAACACTGGCCAGTCGTACTAGC
CCCTTGCGATTGTCAGACGAGCAACAAGCGCTATTAAACTTACCTAAATTTC
TAACCACCACCATTGGTTCTTCACAAACTCAAAAAACTCGTCAAATCCGCTTG
CAATTAAACGCGATGACATAGATCTAACGATTATCAAACCCGATTCAAGCGC
TCATTAAAAACGCACCACTGGCAAGAAGTTCTACCTGCACTGACCAATATGCAAG
CGGCGGCGGAAGAGAGCTGCCTTGATCGATCAAGAAGAAGGGAGCAGCAAAGAGG
AAAACAATCAAAAAGAGGGAGAGCAATCAAATAAAACGAGTTATTGAGGATT
AATTAAACAGGTATATTAAATACCTCTCGTAGTAAACAAATGACTGTATT
CACAAAAATAAAAGAGGTATACCATGTCAAACATCTGGTTGAAGTACCAAAG
ATTGAAGTATTAAACCGTCAAATGGAAAATACTGCCTGCAGCAACTAGGCATT
AAATTACAGAAATTGGCGATGATTATATCACTGGCACAATGCCAGCAGATGCACG
TACCTTCCAGCCAATGGGACTGATTGATGGCGGCTCAAATGTATTGCTGGCAGAA
ACACTGGGCAGCATGGCAGCTAACTGCTGTATTAAATTGTCTCAAGAATATTGTG

TTGGCCAAGAAATTACGCCAACACATACGCGGTGTCGTTCCGGCATAGTGAC
TGGCACAGCAACGCTAGTACACAAAGGAAGAACCTCCCAGATTGGGAAATTG
CATCGTTAACGATCCAAAGAATTCAAAAAGCTCTCGAGAGTACTCTAGAGC
GGGCCCGATCGATTCCACCCGGTGGGTACCAGGTAAGTGTACCCAATT
ATAGTGAGTCGTATTACAATTCACTGGCCGTCGTTAC

Figure 9:

Amino acid sequence of the stabilized single ring mutant Glu461Ala/Ser463Ala/Val464Ala of Cpn60:

SEQ ID No 14: Cpn10 (nucleotides 458-751 of Figure 10):

MKIRPLHDRIVVRRKEEETATAGGIILPGAAAEEKPNQGVVISVGTGRILDNGSVQALA
VNEGDVVVFGKYSGQNTIDIDGEELLILNESDIYGVLEA

SEQ ID No 15: Cpn60 (nucleotides 458-751 of Figure 10):

MAAKDVLFGDSARAKMLVGVNILADA
RVTLGPGRNVVIEKSFGAPIITKDGVSV
AREIELDKFENMGAQMVK
EVASQANDQAGDGTTATVLAQAI
ISEGGLKSVAAGMN
PM^DLKR^GIDKATAAVVAAIKEQA
QPC^LDTKAIAQVGTISANADETV
GRLIAEAMEKV
GKEGVITVEEGK
GLEDEL DVVEGMQFDRGYLSPYFINNQE
KMTVEMENPLILLVDKK
IDNLQELLPILE
NAVKSGRPLL
LIVAEDVEG
QALATLV
VNNLRGTFK
VAAVKAPG
FGD
RRKAMLQDLAILTGGQ
VISEELGMSLETADP
SSLGTASKV
VIDKENTV
VDGAGTEAS
VNTRVDQ
IRAEIESSTSDY
DIEKLQ
ERVA
KLAGGV
AVIKVG
AGSEM
MEMKE
KKDR
VD
DALHAT
RAAVEEG
VVAGGG
VALIR
ALSS
VT
VVG
DNED
QNV
GIAL
ALR
AME
API
RQI
AGN
AGAA
VVD
KV
KSG
TGS
FGFN
AST
GEY
GDM
IAM
GILD
PAK
VTR
SSL
QAA
ASI
AGLM
ITTE
AMV
ADAP
VEEG
AGGG
MPDM
GGM
GGMPGMM

Figure 10:

SEQ ID No 16: DNA sequence of the stabilized single ring mutant

Glu461Ala/Ser463Ala/Val464Ala:

In the DNA sequence:

Small letters – the Cpn10-Cpn60 coding fragment,

Big bold letters = introduced mutations

ggtaacgcaggtgctgCagggGcagCggtgtgataaagtgaatctggcacagtagcttggttaacgccagcacaggtagtatggcgatattgcgatggtagaccctgcaaaagtacgcgttcatctacaagccgcccgtatcgcaggttgatgatcacaaccgaagccatggcgatgcgcgtgtgaagaaggcgctggatgcctgatatggcggcatgggtgaaatggcggtatgcctgatctaccgttaaaaagatcaggctcaaggctgtctataaaaagccgtatcttgcgtgacttagtgcgtttctgctgaaaacgacattggagtgcggctttttgattttgcataaaattcaaatattgtgatatttatgtaactagctggctataatgttgagttcctctgggtggcatgatctcatggtacttcacttaagcctgattcactgcgccttaacagtaataacgcaacgtagaaacataataagcgtatggcattaatgaagacggctgcatttaattcagatc